

## Phenotypic variability in patients with osteogenesis imperfecta caused by BMP1 mutations

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## Phenotypic Variability in Patients with Osteogenesis Imperfecta Caused by BMP1 Mutations

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## Phenotypic Variability in Patients with Osteogenesis Imperfecta Caused by *BMP1* Mutations

Running Title: *BMP1* mutations in Osteogenesis Imperfecta

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ABSTRACT

Osteogenesis Imperfecta (OI) is an inherited bone fragility disorder most commonly associated with autosomal dominant mutations in the type I collagen genes. Autosomal recessive mutations in a number of genes have also been described, including the *BMP1* gene that encodes the mammalian Tolloid (mTLD) and its shorter isoform bone morphogenic protein-1 (BMP1). To date, less than 20 individuals with OI have been identified with *BMP1* mutations, with skeletal phenotypes ranging from mild to severe and progressively deforming. In the majority of patients, bone fragility was associated with increased bone mineral density (BMD), however the full range of phenotypes associated with *BMP1* remains unclear.

Here we describe three children with mutations in *BMP1* associated with a highly variable phenotype: a sibship homozygous for the c.2188delC mutation that affects only the shorter BMP1 isoform and a further patient who is compound heterozygous for a c.1293C>G nonsense mutation and a c.1148G>A missense mutation in the CUB1 domain. These individuals had recurrent fractures from early childhood, are hypermobile and have no evidence of dentinogenesis imperfecta. The homozygous siblings with OI had normal areal BMD by dual energy X ray absorptiometry whereas the third patient presented with a high bone mass phenotype. Intravenous bisphosphonate therapy was started in all patients, but discontinued in two patients and reduced in another due to concerns about increasing bone stiffness leading to chalk-stick fractures. Given the association of *BMP1*-related OI with very high bone material density, concerns remain whether anti-resorptive therapy is indicated in this ultra-rare form of OI.

**KEYWORDS:** Osteogenesis Imperfecta, bone fragility, *BMP1*, Bone morphogenic protein-1, high bone mass.

## INTRODUCTION

Osteogenesis Imperfecta (OI) is a rare inherited connective tissue disorder characterised by an increased tendency to fracture, often with minimal or no apparent trauma. Extra-skeletal features can include short stature, skin and joint hyper-extensibility, blue sclerae, deafness and dentinogenesis imperfecta. Other features are bone pain, deformities, scoliosis and impaired mobility.

Genetic characterisation of families affected with OI has shown that autosomal dominant mutations in the genes that encode the alpha chains of type I collagen (*COL1A1* and *COL1A2*) can be identified in approximately 85-90% of affected individuals [Forlino and Marini 2016]. Mutations in a variety of other genes encoding proteins involved in type I collagen biosynthesis, bone cell differentiation, bone formation and bone remodelling are known to result in rare forms of autosomal recessive OI [Mendoza-Londono et al 2015]. A hallmark of OI at the tissue level is increased bone mineralisation density [Rauch and Glorieux 2004].

Mutations in the *BMP1* gene have been described in a small number of individuals with OI. The *BMP1* gene (OMIM 112264) is alternatively transcribed to produce two proteins, Mammalian Tolloid (mTLD) and its shorter isoform bone morphogenic protein-1 (BMP1). The BMP1/mTLD protein acts as an astacin metalloprotease whose functions include the proteolytic removal of the carboxyl-terminal propeptide from procollagen type I, II and III and the amino-terminal propeptide from types V and XI procollagen. Studies in BMP1/mTLD deficient patients with OI have demonstrated delayed cleavage of type I collagen C-propeptide [Valencia et al 2014] and disorganization of type I/V collagen fibrils as well as impaired processing of the small leucine rich proteoglycan (SLRP) prodecorin [Syx et al 2015].

The OI phenotype of individuals with *BMP1* mutations has been described as recurrent fractures, generalized bone deformity, osteopenia and Wormian bones [Martinez-Glez et al 2012], and also as bone fragility associated with an increase in areal bone mineral density (BMD) as measured by dual-energy X-ray absorptiometry (DXA) [Asharani et al 2012], similar to mutations that affect the C-propeptide cleavage site of type I collagen [Lindahl et al 2011]. At the tissue level, bone of one OI

patient with *BMP1* mutations was found to be even more hypermineralized than in OI caused by collagen mutations [Hoyer-Kuhn et al 2013]. Patients with mutations affecting the C-propeptide cleavage site have also been reported to have bone mineral content that exceeds that of classical OI [Lindahl et al 2011]

However, less than 20 individuals have been described with *BMP1*-related OI and therefore the full range of phenotypes associated with mutations in this gene is not well established. Here we describe three patients that further expand the phenotypic spectrum of *BMP1*-related OI; a sibship presenting with OI and normal BMD and a further patient presenting with a high bone mass phenotype.

MATERIALS AND METHODS

Clinical Information and DNA extraction

Clinical information was obtained from the patients' medical records. The patients and their parents provided informed consent. Total genomic DNA was isolated from 2 to 5 ml peripheral blood taken from patients and parents using standard extraction methods.

DNA sequencing and mutation analysis

Targeted exome sequencing using SureSelect XT (Agilent Technologies) and Illumina MiSeq platform was used to sequence all coding regions and intron/exon boundaries of genes previously described in OI. The variants identified in the *BMP1*/mTLD gene were compared to reference sequences NM\_001199.3 and NM\_006129.4 and their pathogenicity assessed using Alamut Visual version 2.6 (Interactive Biosoftware, Rouen, France).

RESULTS

Clinical Characteristics

**Patient 1**, the first child of consanguineous parents of Asian origin was born at 37 weeks gestation weighing 2.5kg. At 6 years of age, he was referred to the metabolic bone clinic for investigation having sustained 8-10 fractures in total including both tibiae, forearms, and a finger and toe. His first fracture, of the right wrist, occurred at age 12 months. He had no other significant medical

conditions, normal hearing and cognitive development. Due to non-union of a transverse right tibia fracture he had sustained at age 4 years, he mobilised using a wheelchair. On clinical examination there were no dysmorphic features. He had white sclerae, hypermobile fingers and no evidence of dentinogenesis imperfecta but severe dental decay requiring multiple tooth extractions. He had normal serum calcium and phosphate, alkaline phosphatase, parathyroid hormone and vitamin D levels. His lumbar spine BMD Z score, measured by DXA was -0.9. A diagnosis of OI was thought to be likely and intravenous pamidronate treatment (1.5mg/kg/day over 2 days 3 monthly) was commenced on the basis of persistent vertebral compression fracture at L1 (Figure 1) on a background of multiple long bone fractures. The non-union of the long-standing right tibial fracture required intramedullary nailing which allowed better mobility but never united subsequently. Further intramedullary nailing of left tibia and femur was required because of mid-shaft fractures. Pamidronate treatment was paused after 1 year to promote healing of osteotomies. From age 9 years, he received zoledronic acid (0.05mg/kg/day single dose 6 monthly). His bone density increased in response to bisphosphonate therapy (Table I). However he continued to have long bone fractures, including a new vertebral fracture (T10), an oblique subtrochanteric left femur fracture age 11y (Figure 1) and a chalk-stick like mid-shaft femur fracture age 13y, following 7.5 years on bisphosphonate therapy (Figure 2), which was subsequently discontinued.

He is currently growing along the 10<sup>th</sup> centile for height and less than 4<sup>th</sup> centile for weight. His current head circumference is 52.2 cm (age 14 years, between -1 to -2 SD).

His sister, **Patient 2**, was born at 36 weeks gestation weighing 2.42kg. She presented at 7 months of age with bilateral ulnar and radial fractures following a fall down the stairs whilst in the arms of her older sister. Over the following 3 years she had three low impact tibia fractures necessitating right tibial rodding. Zoledronic acid infusions (0.035mg/kg/day single dose 4 monthly) were started at 3 years of age. On clinical examination, she had grey sclerae, hypermobile fingers and no evidence of dentinogenesis imperfecta. She had no other medical conditions, normal hearing and cognitive and gross motor development. Her height and weight are on the 3<sup>rd</sup> centile for age. Her bone profile and



vitamin D level at the time of diagnosis were normal. DXA scanning prior to treatment was not performed due to lack of paediatric bone mineral apparent density (BMAD) reference data and cooperation of children under 5 years [Crabtree et al 2014] . At the age of 5.2 years , her lumbar spine BMAD Z score was 0.9 and total body less head (TBLH) BMD Z score was 1.5, following 2 years of zoledronate treatment during which she sustained one further tibia fracture. Lateral vertebral assessment showed no evidence of vertebral compression fractures. Her head circumference currently is 47.5 cm (age 5.2 years, -2 SD). In light of her increasing BMAD and experience with her brother, zoledronate therapy was reduced in frequency to a once yearly infusion (0.05mg/kg).

**Patient 3**, the only child of healthy non-consanguineous parents of North European origin. She was born following IVF treatment at 39 weeks gestation with a birth weight of 2.976 kg and her early developmental assessments were normal with the exception of gross motor development which was delayed; she sat up at 1 year of age and walked around 17 months of age. She was diagnosed with bilateral dislocated hips at 22 months of age for which she had surgery twice, and was immobilised in the hip spica. The patient's first fracture, of the right fibula, occurred at the age of 2 years and 11 months. She was reviewed at the age of 3yrs and 2 months, having sustained separate fractures of her right fibula and left tibia. Her lumbar spine (L1-4) BMD was 0.726 g/cm<sup>2</sup> (BMAD Z score +4.2,calculated retrospectively ) and did not influence management because lateral thoracic and lumbar spine radiographs done at the same time did not show any vertebral deformity, osteopenia or clear radiological evidence of increased bone density. Her lower-limb fragility fractures were attributed to prolonged immobilisation in hip spica. She was subsequently seen in another centre having had further fractures, including a spiral fracture of the tibia and three metatarsal fractures in the left foot and was empirically treated with pamidronate at age 3¾ years (1mg/kg on three consecutive days, three monthly). She received 4 cycles (total dose 12 mg/kg) before treatment was discontinued as her long bones now appeared abnormally dense on radiographs, and she started to suffer apparent 'chalk stick fractures' of her tibiae & fibulae (Figure 3). At the age of 5½ years, bone mineral density

measurements were undertaken using various imaging techniques (Table II). The girl's distal radial total and trabecular volumetric BMD Z score, measured by peripheral computed tomography, were markedly elevated being +8.7 and +9.2 respectively. At the lumbar spine (LS), her BMAD Z score (+4.3), measured by DXA was elevated but surprisingly not the volumetric trabecular BMD Z score (+0.3) measured by QCT. This apparent discrepancy suggests that the trabecular compartment in the LS is less affected than that at the distal radius, however different reference data used to calculate Z scores by these two techniques in the presence of high cortical bone mass may be a contributing factor. Radiograph of the spine and lateral vertebral fracture assessment by DXA (Figure 3) did not reveal vertebral fractures. A provisional diagnosis of a mild form of osteopetrosis was suggested, however genetic testing for a panel of 21 genes, including *CLCN7* and *LRP5*, was negative. She has continued to have long bone fractures; the most recent are 'chalk-stick' like mid-shaft fractures of the left tibia and fibula at age 7¾ years, 3 years after discontinuation of bisphosphonate therapy.

On clinical examination at aged 7 years, she had white sclerae and normal teeth, hearing and spine. She has a bossed forehead and mild left sided ptosis. She has generalised hypermobility with a Beighton score of 8/9 with soft, velvety and very stretchy skin.

### Identification of *BMP1* mutations

Mutations in the *COL1A1* and *COL1A2* genes were excluded in all 3 patients. Targeted exome sequencing for a panel of additional genes associated with OI revealed that patient 1 and 2 were homozygous for the previously described c. 2188dupC mutation [Syx et al 2015]. The parents were confirmed to be heterozygous carriers.

Patient 3 was compound heterozygous for two novel mutations, a c.1293C>G;p.(Tyr431\*) non-sense mutation and a c.1148G>A;p.(Arg383Gln) missense mutation in the CUB1 domain of *BMP1*. Parental testing demonstrated that the c.1293C>G;p.(Tyr431\*) was present in the mother and c.1148G>A;p.(Arg383Gln) in the father.

## DISCUSSION

To date the majority, but not all, of individuals described with *BMP1*-associated OI have presented with bone fragility associated with increased BMD although no clear genotype-phenotype correlation has yet emerged.

The c.2188dupC identified in patients 1 and 2 is predicted to have different outcomes dependent on the gene transcript. In the shorter *BMP1* transcript, this mutation would lead to the creation of an extended protein (p.Gln730Profs\*294), whereas in the longer mTLD transcript this mutation is predicted to result in an intronic duplication (c.2108-605dupC). Two individuals who are compound heterozygous for this change and the recurring signal peptide mutation, p.(Gly12Arg), have previously been described [Syx et al 2015]. These individuals are reported to have a severe progressive form of OI. Patient 1 and patient 2 presented with a phenotype suggesting that the mutant protein may have residual C-propeptide cleavage activity and the c.2188dupC may therefore represent a relatively 'mild' mutation.

The markedly increased bone mass and 'chalk-stick' pattern of long-bone fractures of patient 3 initially suggested a diagnosis of osteopetrosis. To date, similar compound heterozygous changes that result in a 'null' allele and a mutation in a CUB domain have been associated with severe OI phenotypes. Interestingly, a patient with a c 925delC frameshift mutation and a p.(Gly498Arg) substitution in the CUB2 domain is reported to have severe rhizomelic deformities, short stature and to have sustained over 100 fractures [Syx et al 2015]. Unfortunately no data is available for the associated BMD in this patient but, in contrast to patient 3, extensive skeletal surveys showed generalized undermineralization of long bones.

It remains unclear why some *BMP1* mutations are associated with increased BMD and others with normal or reduced BMD. Areal BMD measurements provide a composite value for bone mass within a given area, and do not reflect tissue mineralisation density – the combination of increased bone material density with reduced bone mass (as is typical in OI) can give values for BMD that sit within the normal range for age in children. However, this is clearly not the case for patient 3, where size

corrected LS BMD (BMAD) and distal radius volumetric BMD are elevated (table II). Mineral crystals in OI patients are known to be smaller, have high calcium content and are more densely packed than in normal bone. Tissue mineralisation density may be a reflection of the degree of matrix disorganisation; some of the highest values are in type VI OI, where patients have a severely disrupted lamellar structure [Land et al 2007]. The multiple potential effects on matrix organisation resulting from mutations in *BMP1* could be similarly disruptive.

Bone tissue analysis of trabecular and cortical bone from an individual homozygous for the *BMP1* p.(Gly12Arg) signal peptide mutation demonstrated increased regions of unmineralised matrix at sites of new bone formation, possibly caused by a delay in matrix maturation necessary for mineralisation. In contrast, hypermineralisation was observed at older bone sites in the same bone sample, hypothesised to result from an increase in matrix space caused by retention of the C-propeptide in collagen fibrils which is subsequently filled by mineral crystals [Hoyer-Kuhn et al 2013].

Functional studies in patients with *BMP1* mutations have largely focused on C-propeptide cleavage activity. However, BMP1/mTLD is also involved in processing of additional extra cellular matrix components, in particular the processing of the SLRP prodecorin by removal of the prodomain, which has been shown to be delayed in patients with *BMP1* mutations [Syx et al 2015]. Decorin is known to influence both collagen assembly and regulate matrix mineralization [Mochida et al 2009]. The CUB domains of BMP1/mTLD are essential for C-proteinase activity; thus, mutations in different CUB domains may also be contributing to the variation in mineralization seen in these patients through their interaction with SLRPs. Potentially, this may explain why our patients with the c.2188dup, where all the CUB domains are intact, did not present with a high bone mass phenotype.

Intravenous bisphosphonate therapy was started in all patients, but discontinued in two patients and reduced in the younger sibling due to concerns about increasing bone stiffness contributing to occurrence of chalk-stick fractures. Whilst bisphosphonates are not known to increase bone material density or stiffness in OI caused by collagen gene mutations [Weber et al 2006], they impair bone

remodelling/repair and healing, possibly allow accumulation of microdamage [Chapurlat and Delmas 2009] and are linked to atypical fractures in adults [Shane et al 2014]. Given the very high bone material density associated with *BMP1*-related OI and the potential risk of causing delayed healing, increased stiffness, atypical fractures [Vasanwala et al 2016] or even iatrogenic osteopetrosis [Whyte et al 2003], concerns remain whether anti-resorptive therapy is indicated in this ultra-rare form of OI.

**Conclusion**

Our patients demonstrate that bone mass in *BMP1*-related OI is highly variable and that OI should be considered as a possibility in individuals presenting with high bone mass and a significant fracture history. In addition, careful consideration and monitoring of response to bisphosphonate therapy in these patients is recommended. As further mutations are identified the functional consequence of *BMP1* mutations and *COL1A1*/*COL1A2* mutations affecting the type I collagen C-propeptide cleavage site will become clearer.

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**Conflicts of Interest:** None to declare

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**Figure legends**

Figure 1: Patient 1 X-rays A. Compression fracture at L1 at age 8.5 years. B. Left low impact subtrochanteric femur fracture at age 11y (after 4.5y on bisphosphonate therapy).

Figure 2: Patient 1 X-rays, age 13y: Left ‘chalk-stick’ mid-shaft femur fracture following a fall at school (after 7.5years on bisphosphonate therapy). Note the mild Erlenmeyer-shape deformity of distal femur with ‘bisphosphonate’ lines.

Figure 3: Patient 3 X-rays. A. ‘Chalk stick’ fractures through right mid-tibia & mid-fibula, with soft tissue swelling. Note dense & thickened cortices. Three ‘Pamidronate lines’ are visible at proximal and distal tibial metaphyses. B. Lateral vertebral fracture assessment by DXA at 5.5 years of age. Note absence of vertebral compression fractures.

**Table I** Response in lumbar spine (LS) and total body less head (TBLH) bone density Z-scores (by DXA) during intravenous bisphosphonate therapy in patient 1. Treatment was paused at age 7 due to rodding surgery.

Age in years	6 Pre- Treatment	7 Treatment paused	8 Treatment recommenced	9	10	12	14
LS BMD	-0.9	-0.8	-1.1	-0.8	-0.4	0.3	0.3
LS BMAD (L1 –L4)	-0.6	-0.6	-0.6	0.4	1.3	2.1	1.8
TBLH BMD	NA	-0.5	-1.7	-0.4	-0.8	0.3	-0.4

LS BMD – lumbar spine bone mineral density, LS BMAD - Lumbar spine bone mineral apparent density, TBLH – total body less head



**Table II:** Volumetric Bone Mineral Density Z-scores of Patient 3 measured by peripheral quantitative computed tomography (distal radius), DXA (lumbar spine) and quantitative computer tomography (lumbar spine). Pamidronate was started at 3.8 years of age and she remained on treatment for 12 months before identification of raised BMD led to treatment discontinuation

Age in years	3.2	5.5	6.5	7
Distal radial total volumetric BMD	Not measured	+8.7	+7.1	+7.2
Distal radial trabecular volumetric BMD	Not measured	+9.2	+6.9	+6.6
LS BMAD (L1 –L4)	+4.2	+4.3	+3.1	+3.4
LS volumetric trabecular BMD (L1 –L3)	Not measured	+0.35	Not measured	Not measured

BMD – Bone mineral density, LS – lumbar spine, BMAD - bone mineral apparent density



Figure 1: Patient 1 X-rays A. Compression fracture at L1 at age 8.5 years. B. Left low impact subtrochanteric femur fracture at age 11y (after 4.5y on bisphosphonate therapy).

111x127mm (150 x 150 DPI)



Figure 2: Patient 1 X-rays, age 13y: Left 'chalk-stick' mid-shaft femur fracture following a fall at school (after 7.5years on bisphosphonate therapy). Note the mild Erlenmeyer-shape deformity of distal femur with 'bisphosphonate' lines.

116x125mm (150 x 150 DPI)



Figure 3: Patient 3 X-rays. A. 'Chalk stick' fractures through right mid-tibia & mid-fibula, with soft tissue swelling. Note dense & thickened cortices. Three 'Pamidronate lines' are visible at proximal and distal tibial metaphyses. B. Lateral vertebral fracture assessment by DXA at 5.5 years of age. Note absence of vertebral compression fractures.

95x126mm (150 x 150 DPI)